Approcci structure based (SB) su inibitori non covalenti del kirsten rat sarcoma viral oncogene homolog (KRAS)

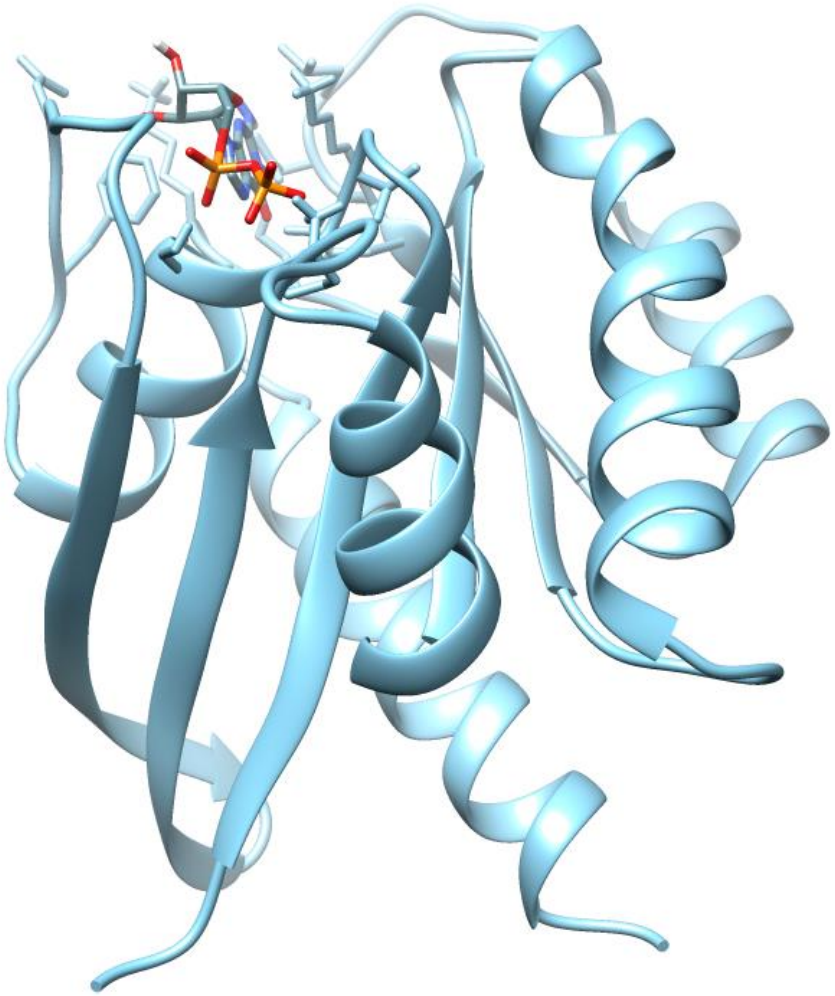
SAPIENZA
UNIVERSITÀ DI ROMA

Facoltà di Farmacia e Medicina
Corso di Farmacia
Tesi Sperimentale in Chimica Farmaceutica
a.a. 2015/2016

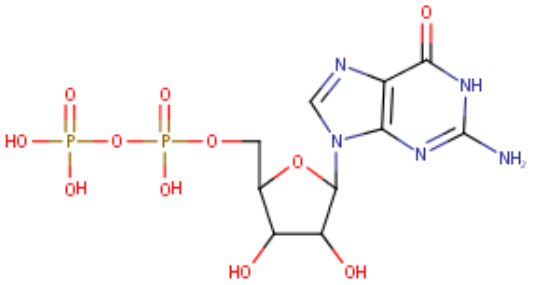
Laureando: Lucia Guida
Matricola: 1352872

Relatore: prof. Rino Ragno

PROTEINE RAS



- GTPasi monomeriche
- 21 kDa
- Scambiatori molecolari



GDP

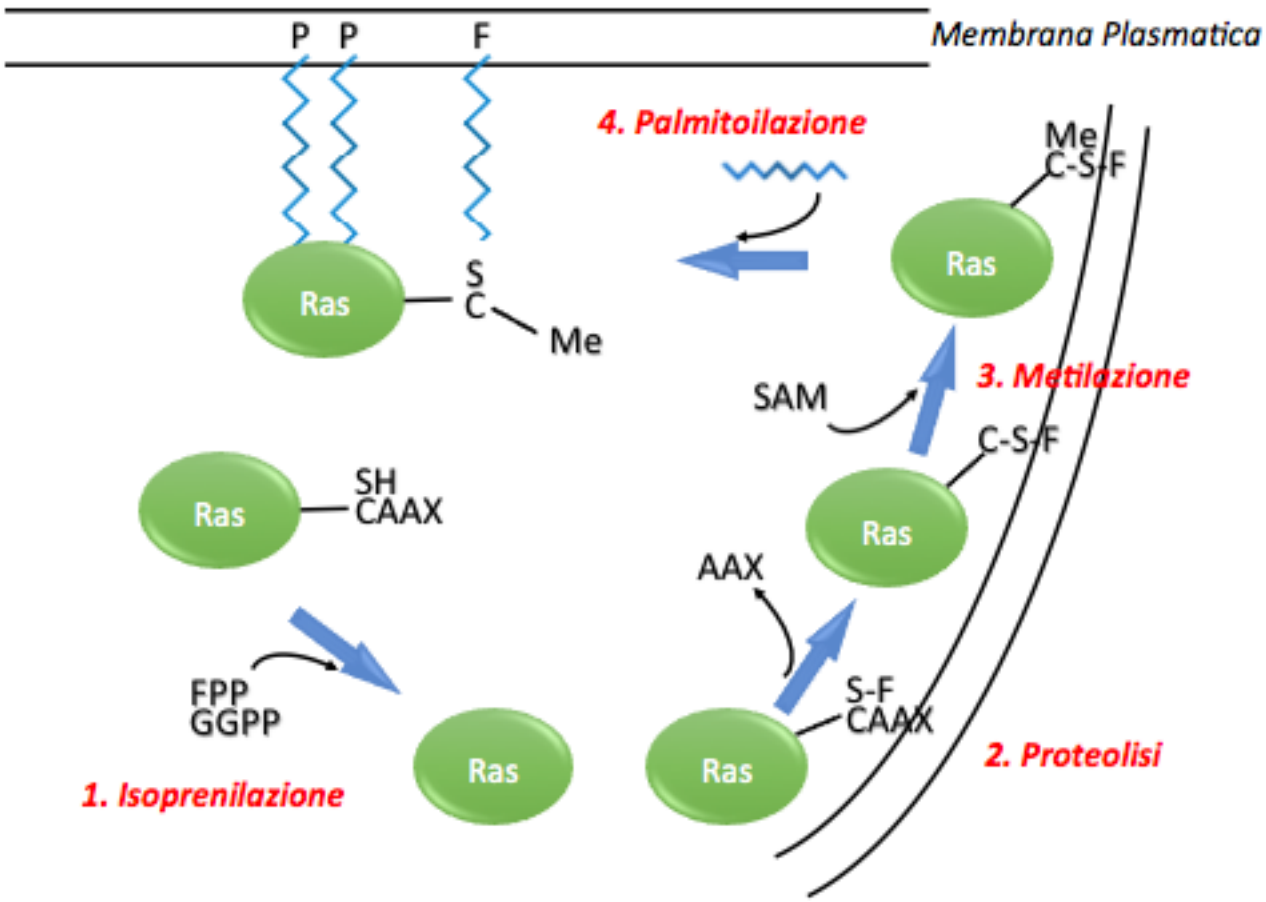
Splicing alternativo

H-Ras
N-Ras → 188 aa
K-Ras4A

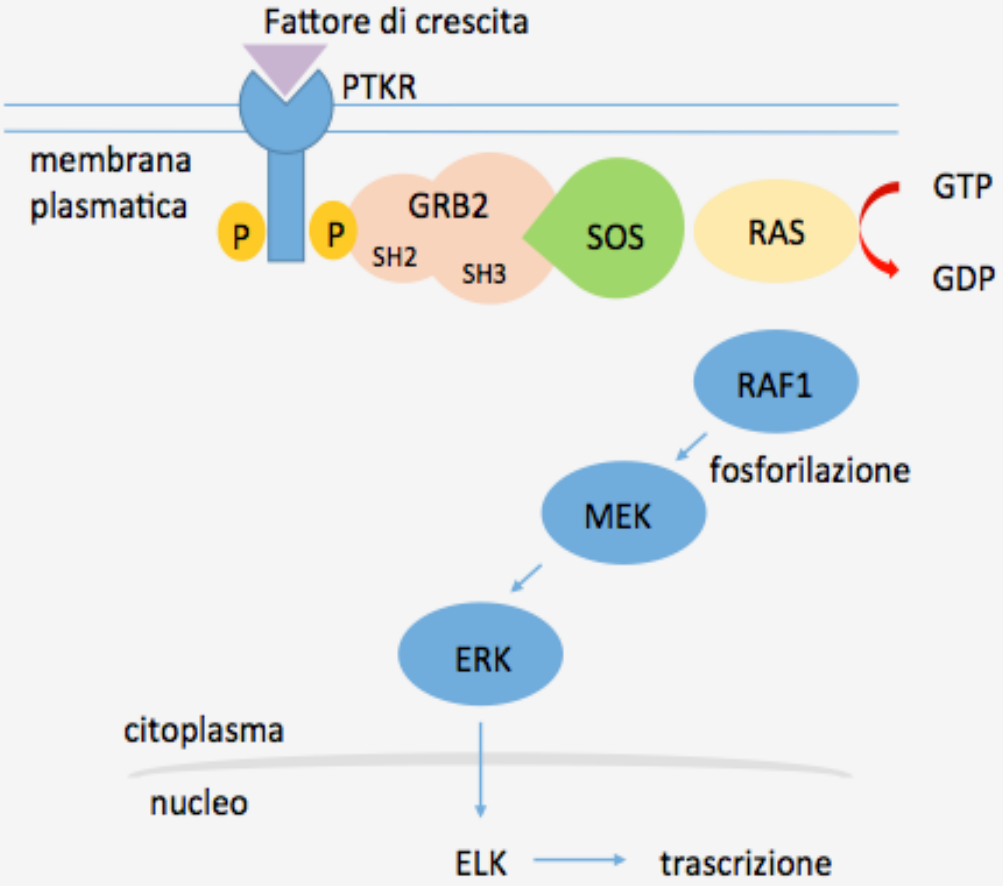
K-Ras4B → 189 aa

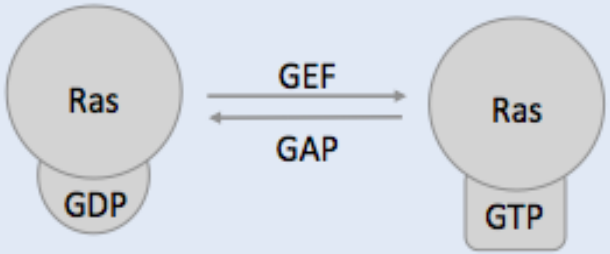
- Effector binding
- GTP binding
- Hypervariable domain
- Switch I
- Switch II

MODIFICHE POST-TRASDUZIONALI



ATTIVAZIONE/INATTIVAZIONE



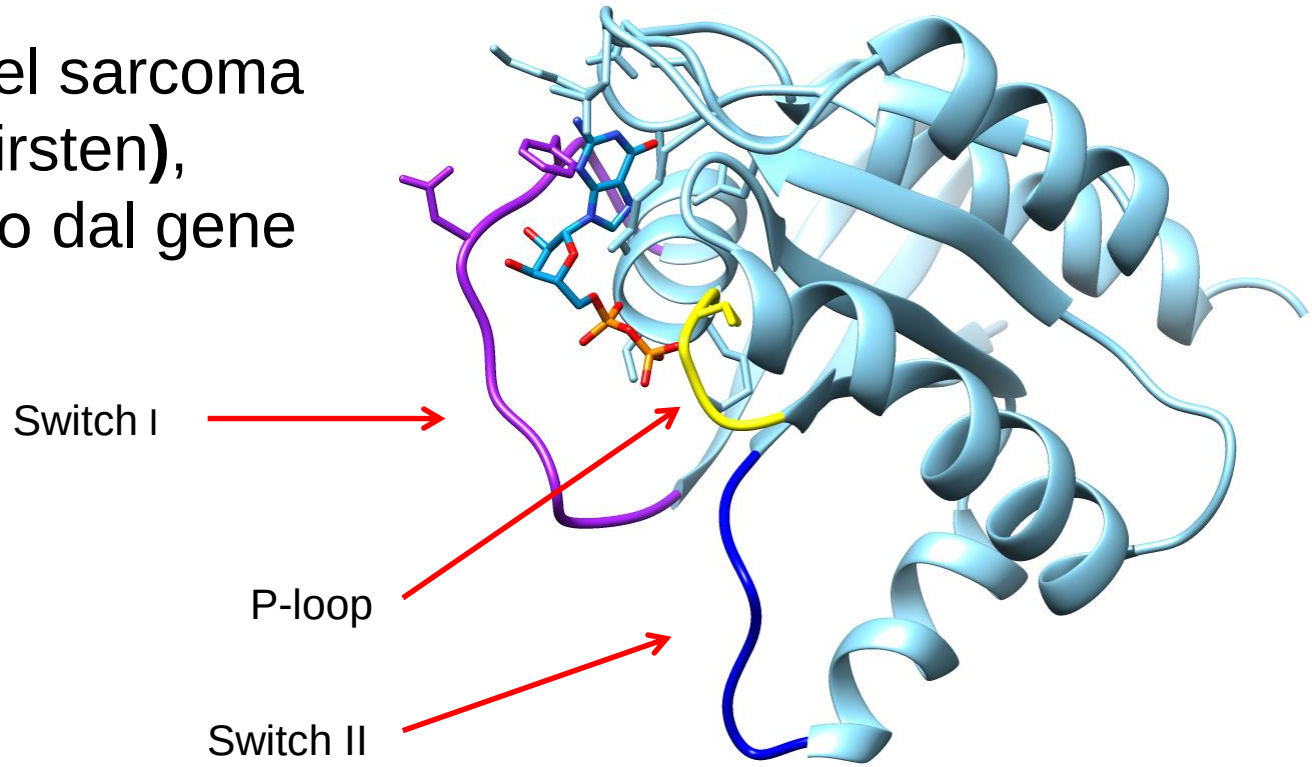


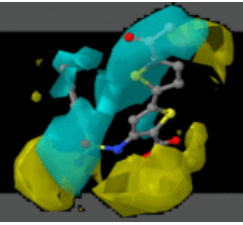
GAPs: SYN GAP, p120, NF1, RASAL, CAPRO, GAP

GEFs: RASGRF, RASGRP, SOS

Effectors: RASSF, TIAM1, RAF1, PI3K, RALGDS, PLCε, RIN1

K-Ras è una GTPasi conosciuta anche come V-Ki-RAS2 (oncogene virale del sarcoma 2 nel ratto di Kirsten), codificata nell'uomo dal gene *kras.*





MUTAZIONI

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Gene	Codone	Analita	Mutazioni rilevate		
KRAS	12	G12A	Gly12Ala	c.35G>C	GGT>GCT
		G12C	Gly12Cys	c.34G>T	GGT>TGT
		G12D	Gly12Asp	c.35G>A	GGT>GAT
		G12F	Gly12Phe	c.34_35GG>TT	GGT>TTT
		G12R	Gly12Arg	c.34G>C	GGT>CGT
		G12S	Gly12Ser	c.34G>A	GGT>AGT
		G12V	Gly12Val	c.35G>T	GGT>GTT
	13	G13A	Gly13Ala	c.38G>C	GGC>GCC
		G13C	Gly13Cys	c.37G>T	GGC>TGC
		G13D	Gly13Asp	c.38G>A	GGC>GAC
		G13R	Gly13Arg	c.37G>C	GGC>CGC
		G13S	Gly13Ser	c.37G>A	GGC>AGC
		G13V	Gly13Val	c.38G>T	GGC>GTC
	61	Q61E	Gln61Glu	c.181C>G	CAA>GAA
		Q61H1	Gln61His	c.183A>C	CAA>CAC
		Q61H2	Gln61His	c.183A>T	CAA>CAT
		Q61K	Gln61Lys	c.181C>A	CAA>AAA
		Q61L	Gln61Leu	c.182A>T	CAA>CTA
		Q61P	Gln61Pro	c.182A>C	CAA>CCA
		Q61R	Gln61Arg	c.182A>G	CAA>CGA

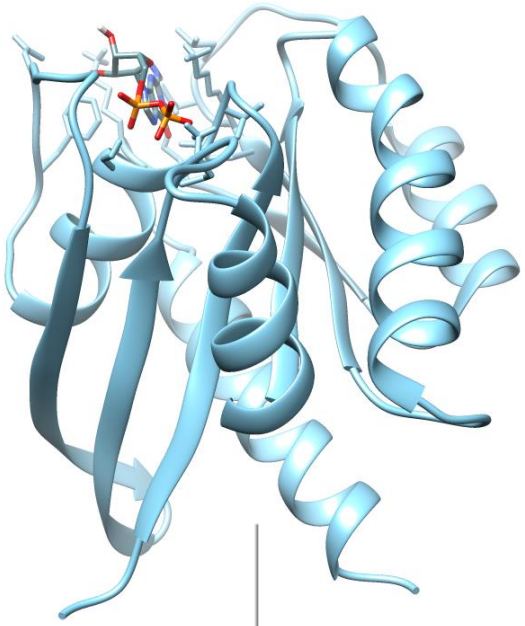
SCOPO

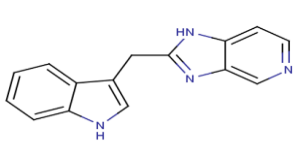
- ADENOCARCINOMA PANCREATICO
- CARCINOMA COLON - RETTO
- CARCINOMA POLMONARE
- LEUCEMIE

CREAZIONE DI NUOVI MODELLI

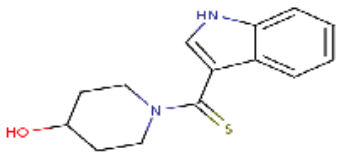
PREDIZIONE ATTIVITA' DI NUOVI COMPOSTI

LIGANDI CO-CRISTALLIZZATI





4EPV



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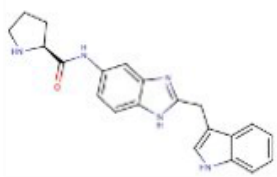

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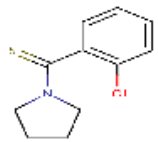

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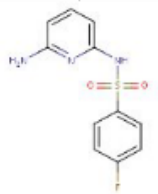

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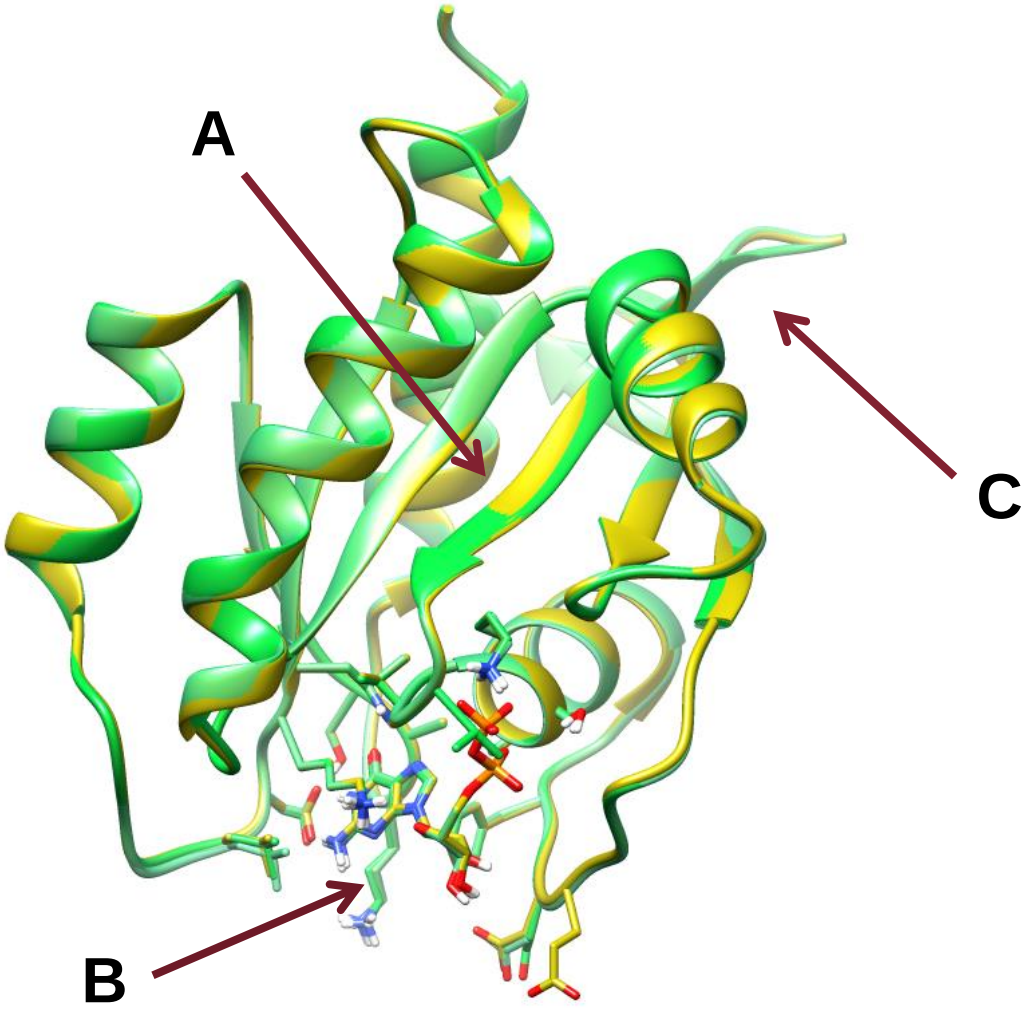

A:
Sito allosterico
“Covalente”

B:
Sito attivo (GDP)

C:
Sito allosterico
sia “non
covalnte
che “covalente”

INIBITORI NON COVALENTI

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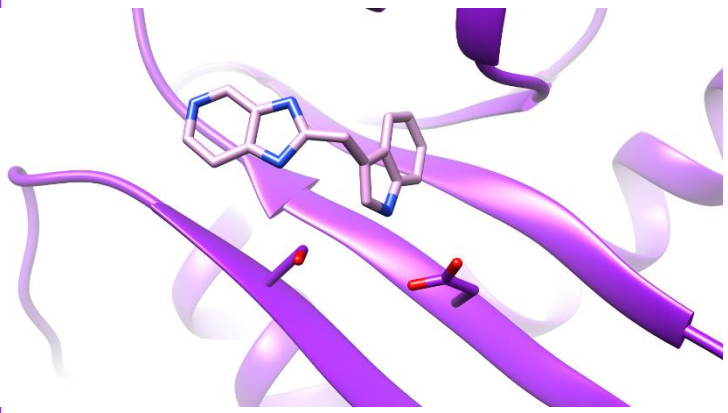
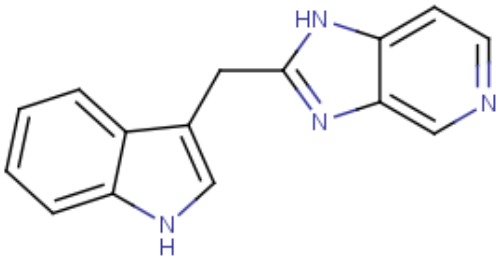
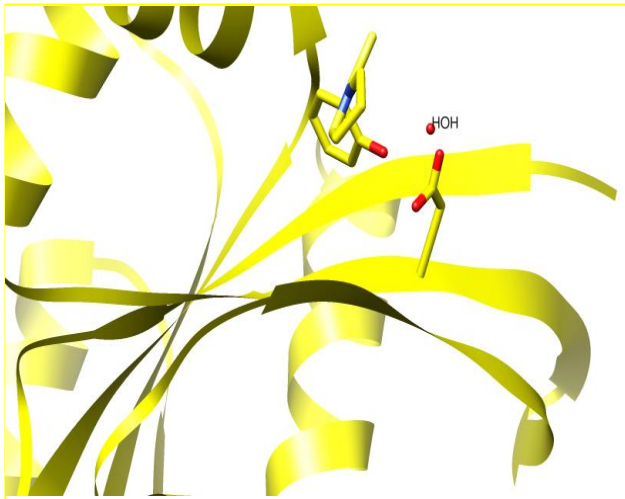
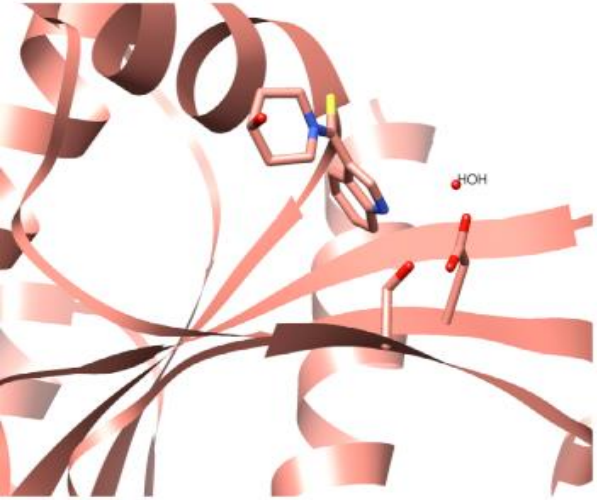
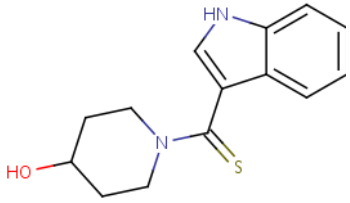




4EPV







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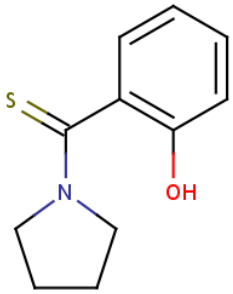

4EPT

FASE SPERIMENTALE

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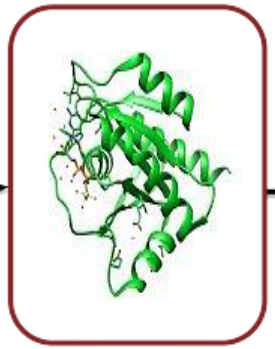
Download dei PDB



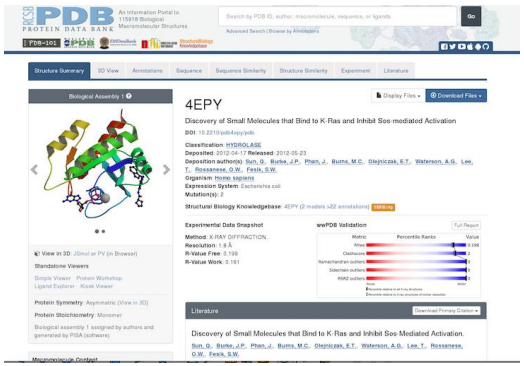
Minimizzazione allineamento

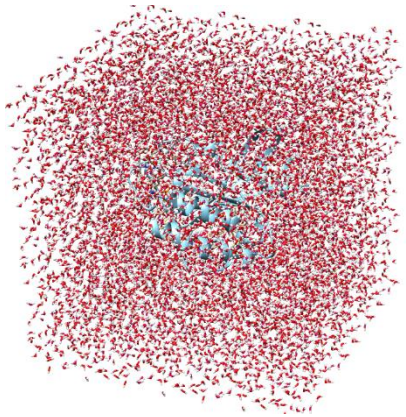
Docking Assessment

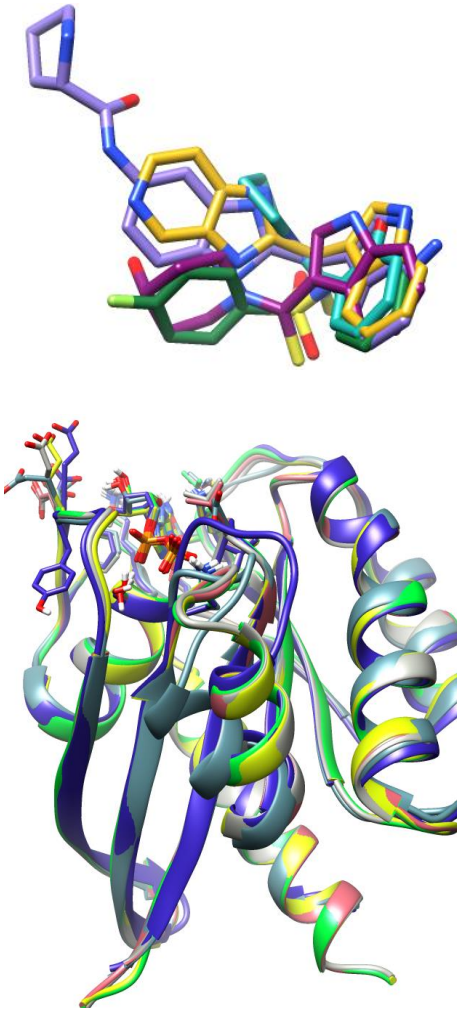
Docking non covalente

Modello preliminare
- 3-D QSAR
- COMBINE

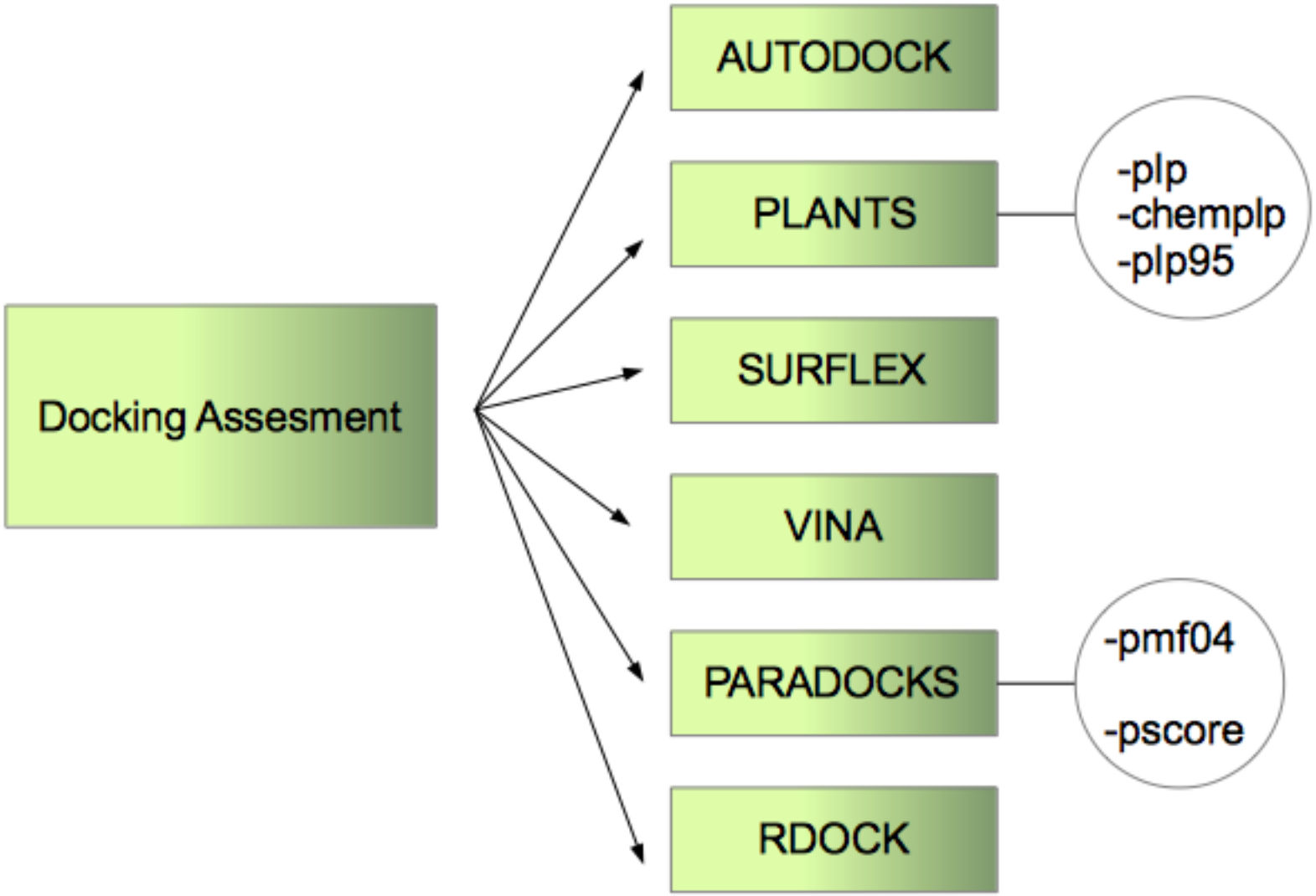
FASE SPERIMENTALE

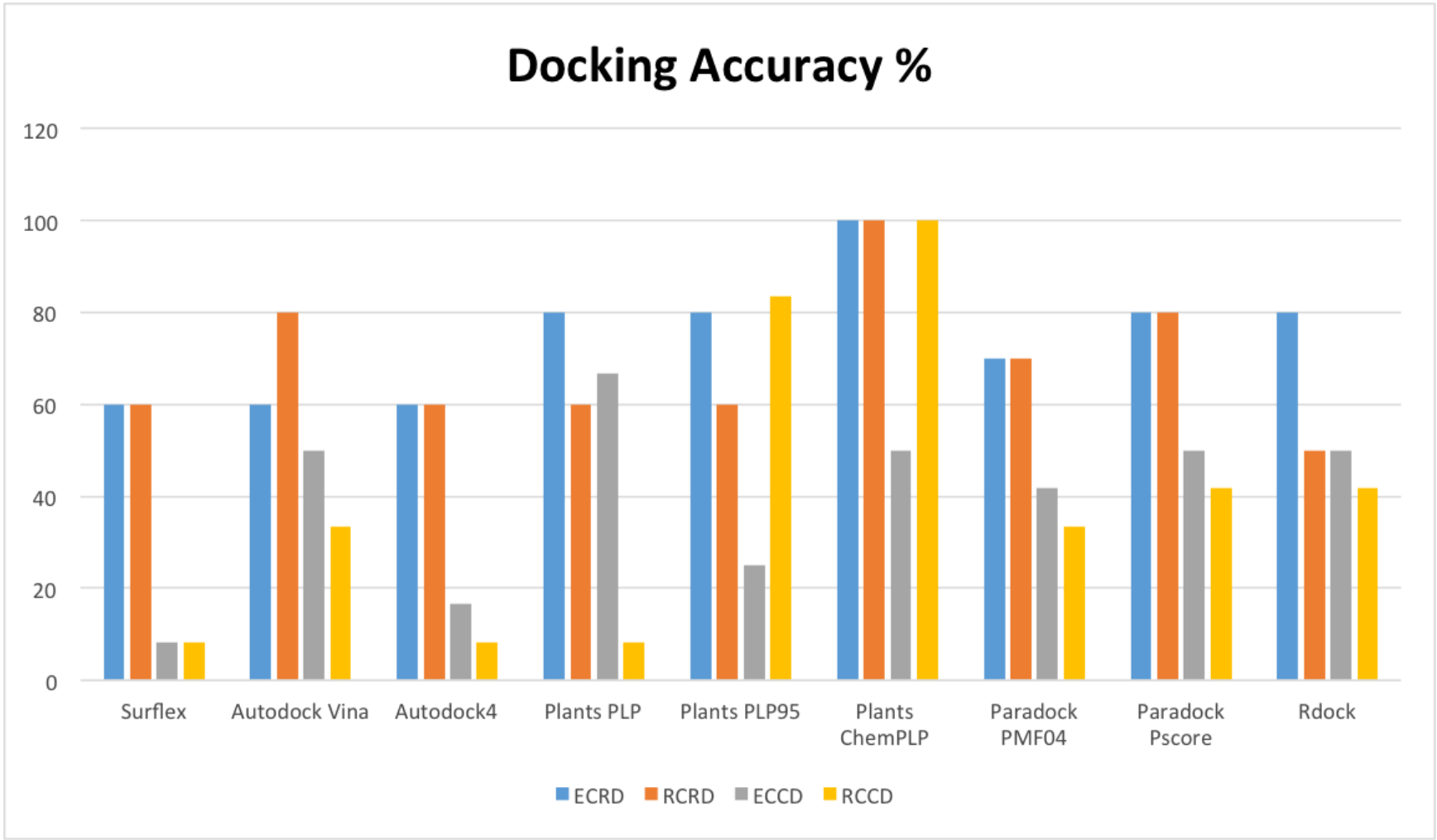






DOCKING MOLECOLARE




Docking Accuracy %
120
100
80
60
40
20
0
Surflex
Autodock Vina
Autodock4
Plants PLP
Plants PLP95
Plants ChemPLP
Paradock PMF04
Paradock Pscore
Rdock
ECRD
RCRD
ECCD
RCCD

APPLICAZIONE DOCKING

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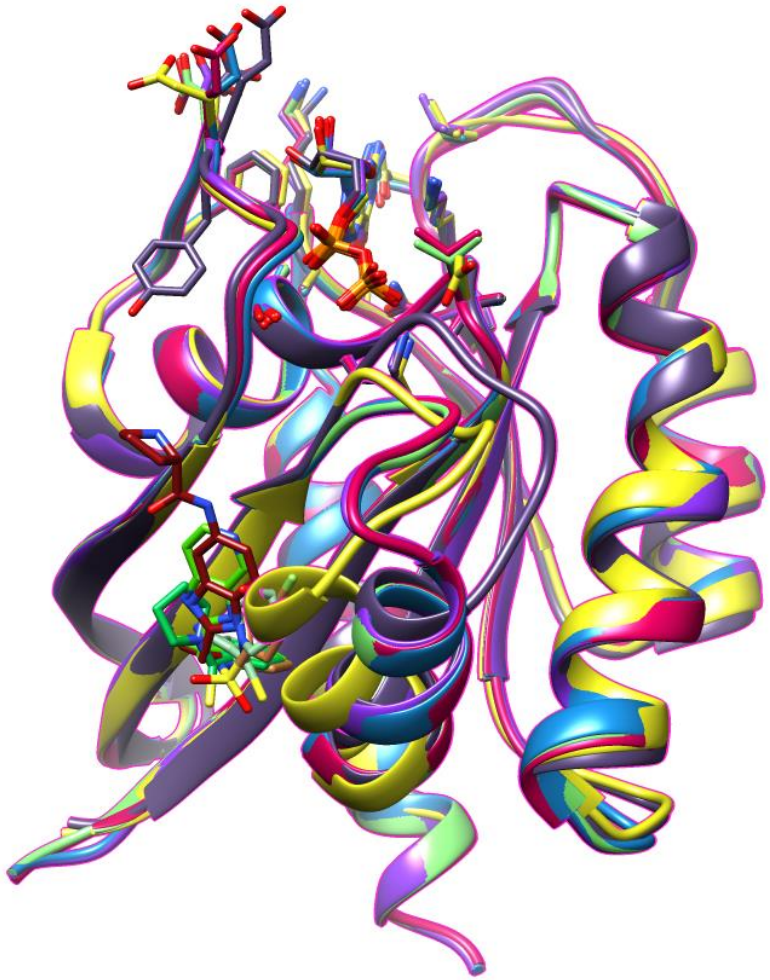
- Analisi DA%
- Plants
- Paradocks
- Cross-docking
- Clusterizzazione
- 3-D QSAR
- COMBINE



KRAS

Training Set
ATTIVITA' BIOLOGICA : pIC_{50}

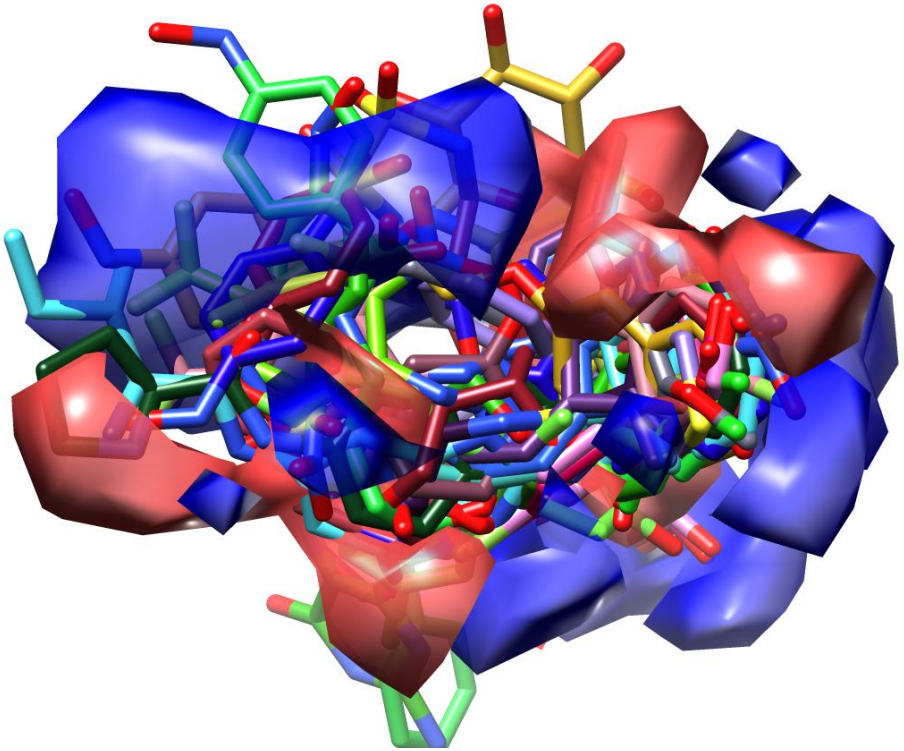
Calcolo dei MIF
(campi di interazione molecolare)

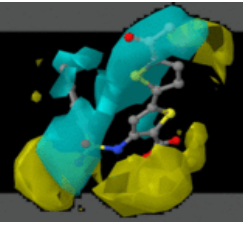
Modello 3-D QSAR preliminare

Validazione Interna

LOO | **Y-Scrambling**

Mappe Tridimensionali





3-D QSAR

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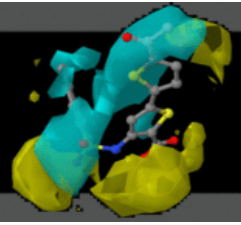
TRAINING SET

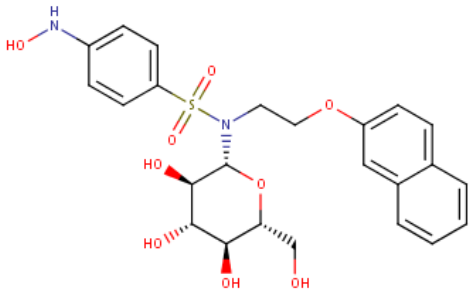
INIBITORE	ATTIVITA' (pIC_{50})
1	3.72
2	3.47
3	4.14
4	4.24
5	4.45
6	4.21
7	5.00
8	3.67
9	3.74
10	4.00
11	3.79
12	3.66
13	3.35
14	5.52
15	3.46
16	3.22
17	6.15
18	5.70
19	4.00
20	5.52

PROBE	PC	r^2	q^2 LOO	q^2 YS
A	5	0,930	0,433	-0,641
C	5	0,930	0,434	-0,609
HD	4	0,986	0,436	-0,534
NA	5	0,922	0,446	-0,568
N	5	0,952	0,443	-0,461
OA	5	0,924	0,435	-0,510
e	5	0,903	-0,171	-0,814
d	4	0,916	-0,368	-1,973



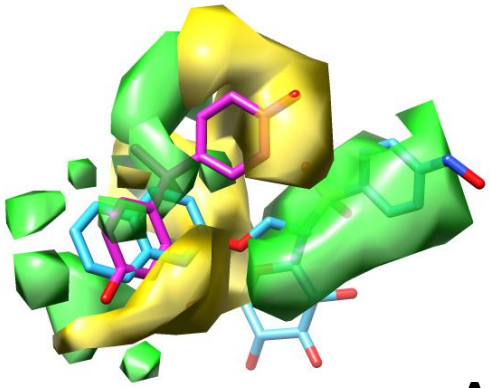
MODELLO PRELIMINARE 3-D QSAR: RAPPRESENTAZIONE GRAFICA

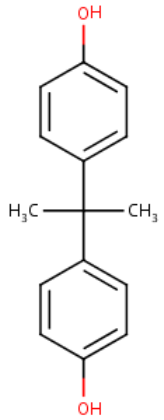
PROBE C



pIC_{50} =6.15

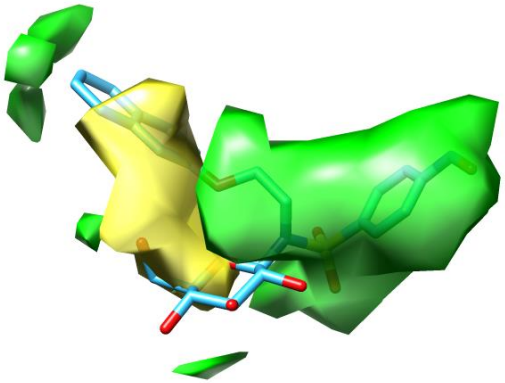
STUDIO MAPPE COMFA2



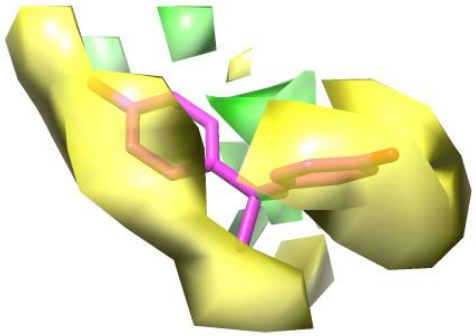


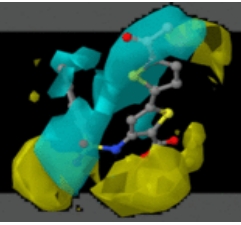
pIC_{50} =3.22

ACTIVITY CONTRIBUTION 17



ACTIVITY CONTRIBUTION 16





MODELLO PRELIMINARE 3-D QSAR: RAPPRESENTAZIONE GRAFICA

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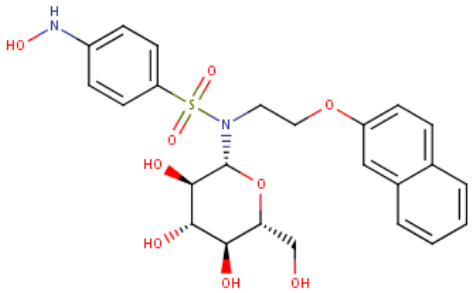
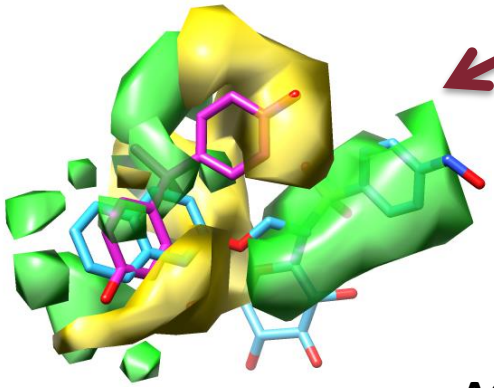
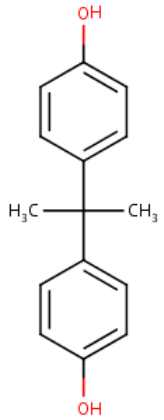
PROBE C



pIC_{50} =6.15

STUDIO MAPPE COMFA2





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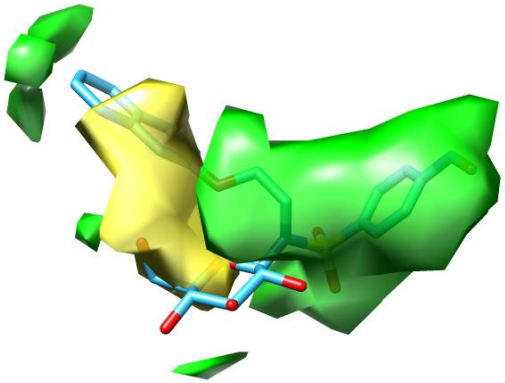
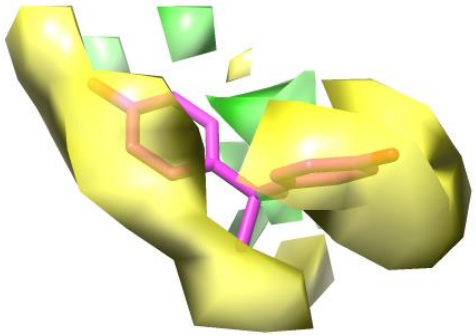
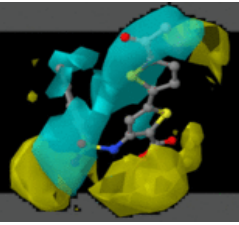
ACTIVITY CONTRIBUTION 17



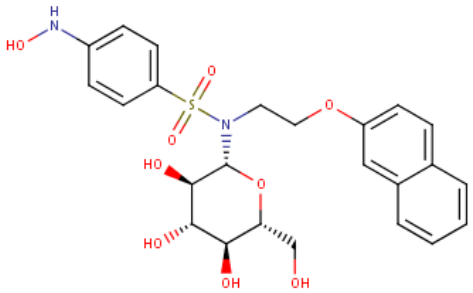
ACTIVITY CONTRIBUTION 16





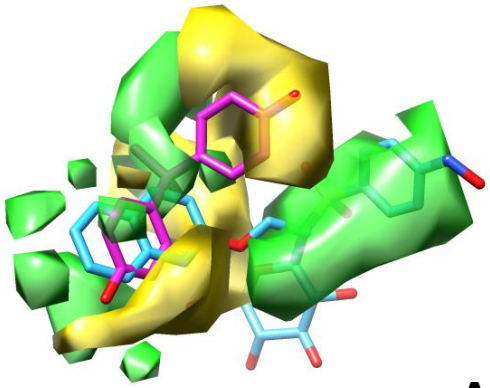
MODELLO PRELIMINARE 3-D QSAR: RAPPRESENTAZIONE GRAFICA

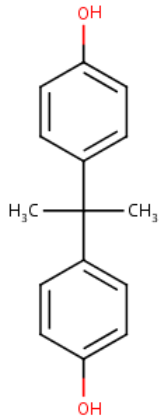
PROBE C



pIC_{50} = 6.15

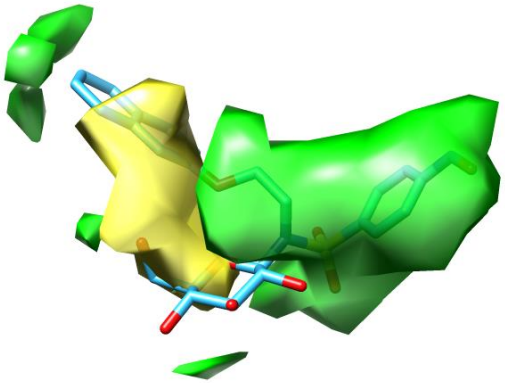
STUDIO MAPPE COMFA2



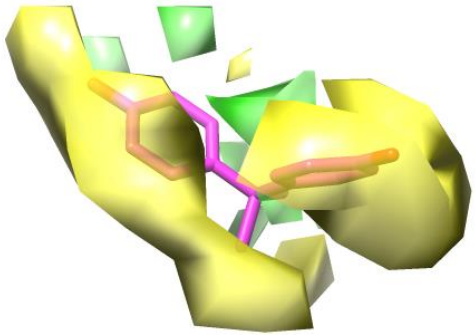


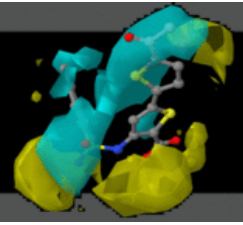
pIC_{50} = 3.22

ACTIVITY CONTRIBUTION 17



ACTIVITY CONTRIBUTION 16





MODELLO PRELIMINARE 3-D QSAR: RAPPRESENTAZIONE GRAFICA

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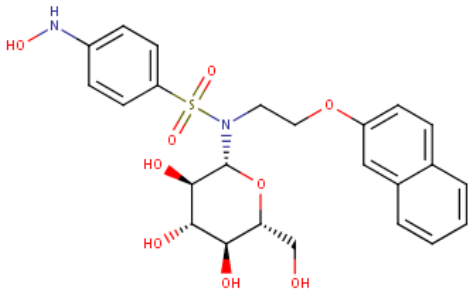
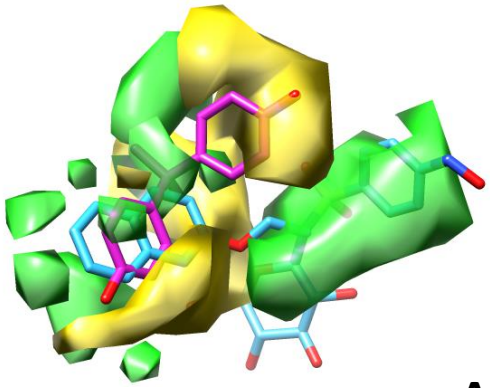
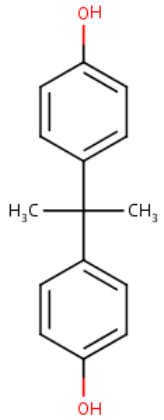
PROBE C



pIC_{50} = 6.15

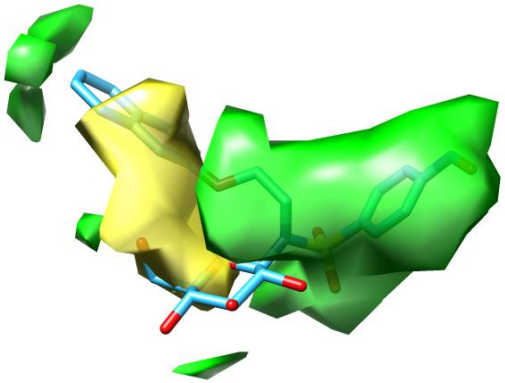
STUDIO MAPPE COMFA2



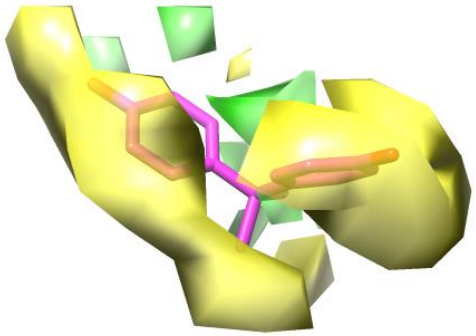


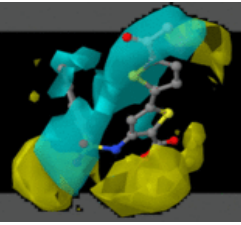
pIC_{50} = 3.22

ACTIVITY CONTRIBUTION 17



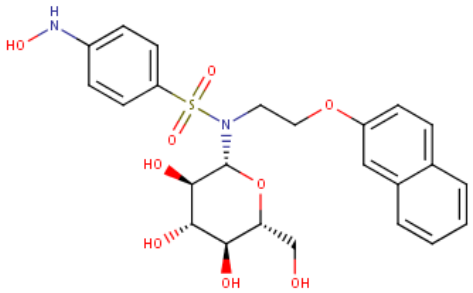
ACTIVITY CONTRIBUTION 16





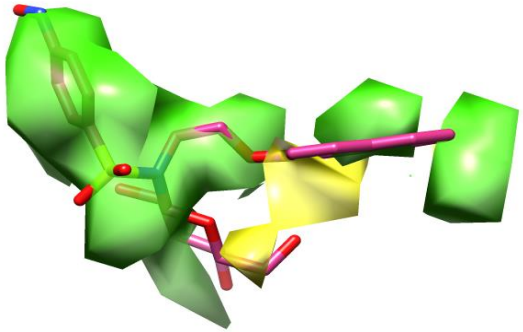
MODELLO PRELIMINARE 3-D QSAR: RAPPRESENTAZIONE GRAFICA

PROBE HD

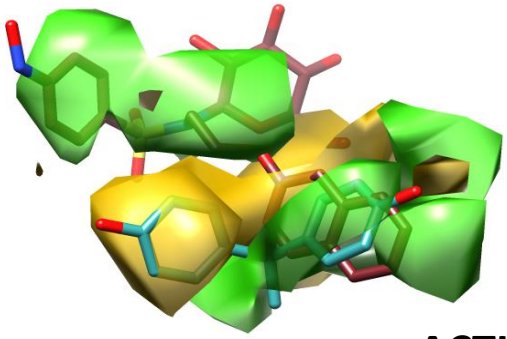


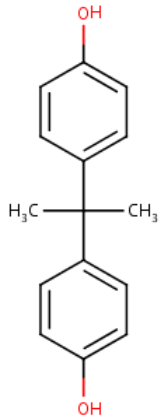
pIC_{50} = 6.15

ACTIVITY CONTRIBUTION 17



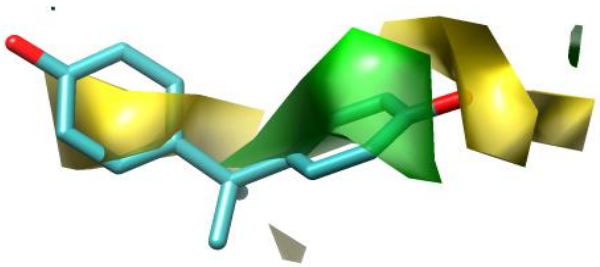
STUDIO MAPPE COMFA2

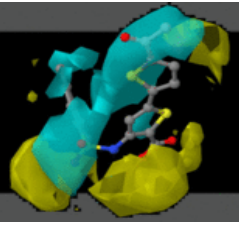




pIC_{50} = 3.22

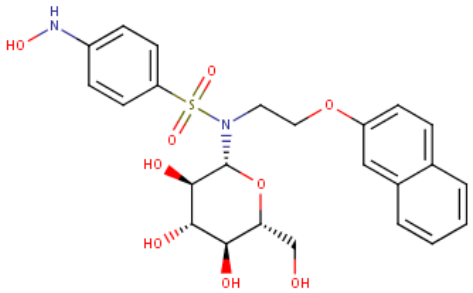
ACTIVITY CONTRIBUTION 16





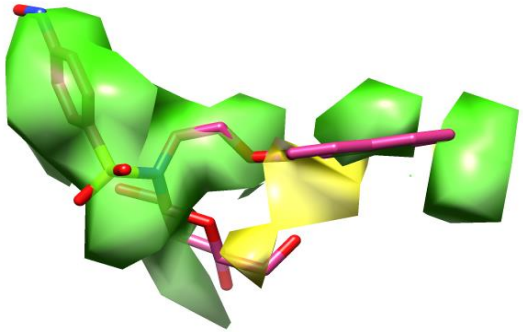
MODELLO PRELIMINARE 3-D QSAR: RAPPRESENTAZIONE GRAFICA

PROBE HD

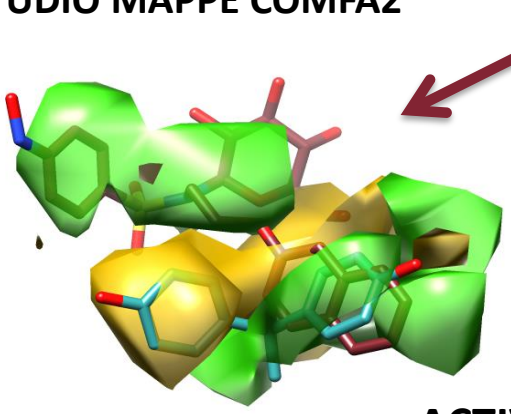


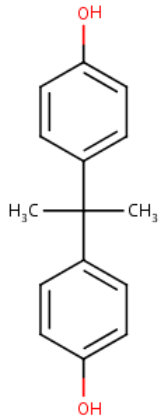
pIC$_{50}$ =6.15

ACTIVITY CONTRIBUTION 17



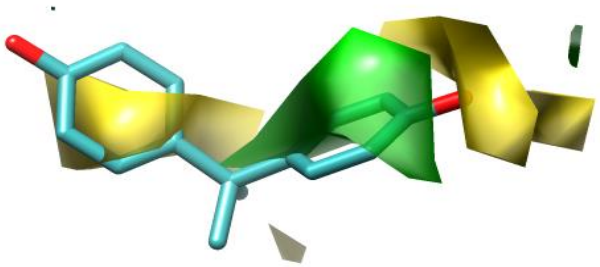
STUDIO MAPPE COMFA2

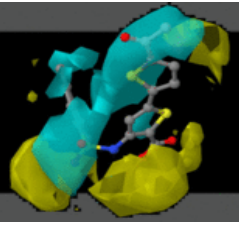




pIC$_{50}$ =3.22

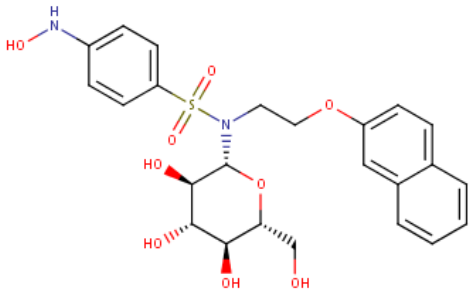
ACTIVITY CONTRIBUTION 16





MODELLO PRELIMINARE 3-D QSAR: RAPPRESENTAZIONE GRAFICA

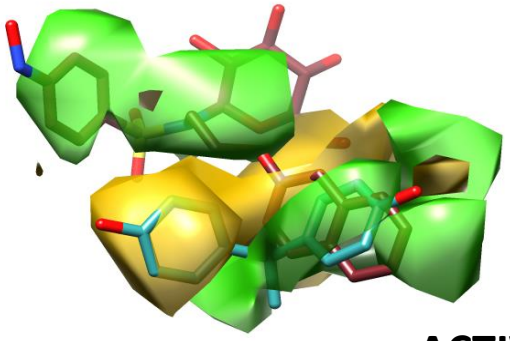
PROBE HD

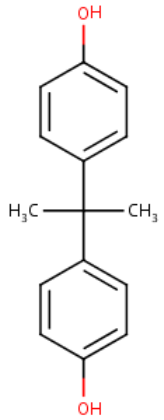


pIC_{50} =6.15

ACTIVITY CONTRIBUTION 17

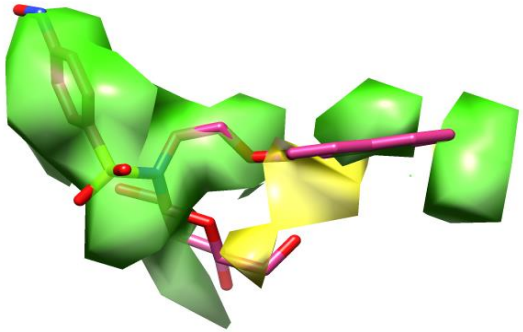
STUDIO MAPPE COMFA2

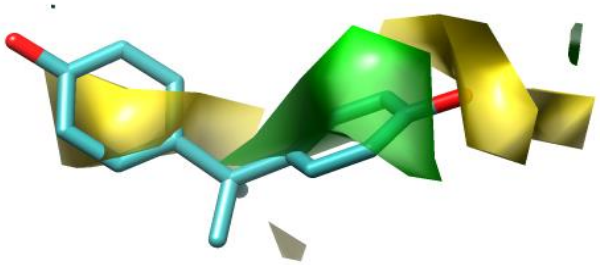


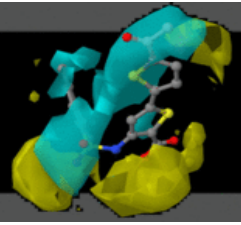


pIC_{50} =3.22

ACTIVITY CONTRIBUTION 16

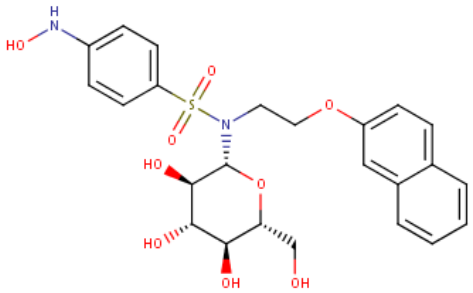






MODELLO PRELIMINARE 3-D QSAR: RAPPRESENTAZIONE GRAFICA

PROBE HD



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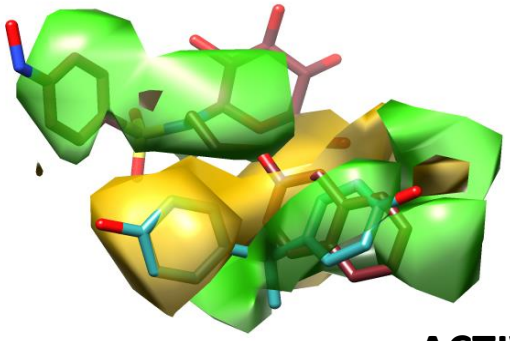
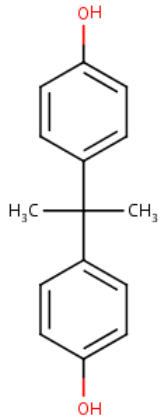
STUDIO MAPPE COMFA2





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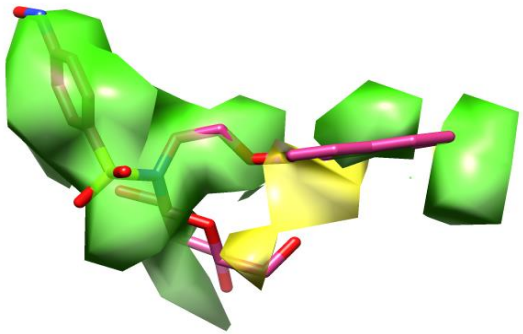

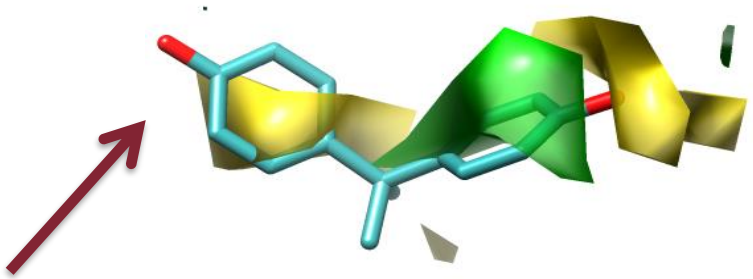

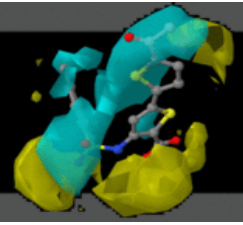

COMBINE

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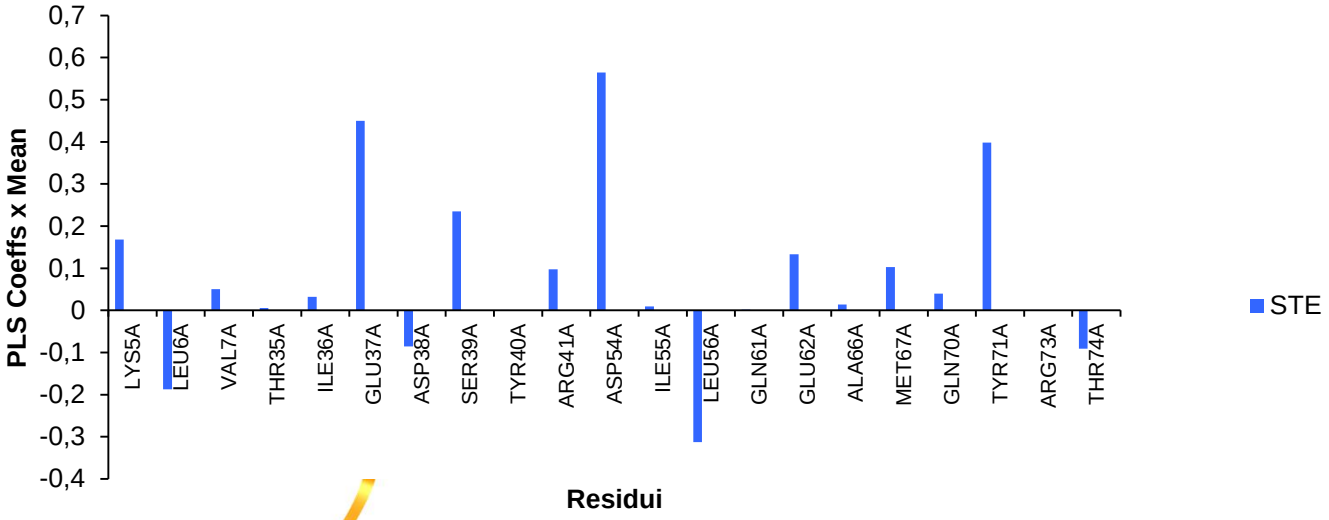
TRAINING SET

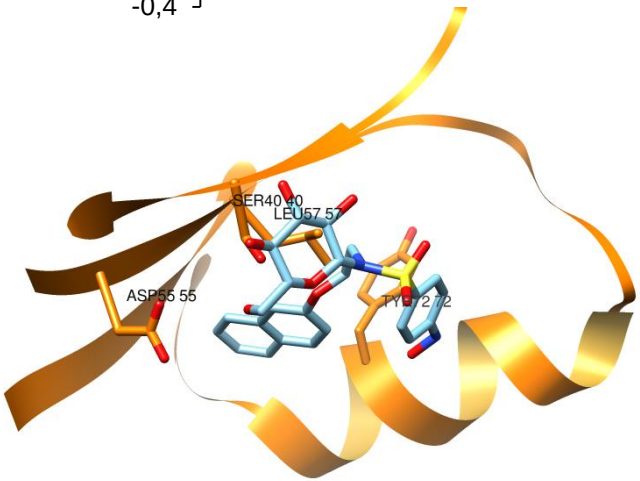
INIBITORE	ATTIVITA' (pIC_{50})
1	3.72
2	3.47
3	4.14
4	4.24
5	4.45
6	4.21
7	5.00
8	3.67
9	3.74
10	4.00
11	3.79
12	3.66
13	3.35
14	5.52
15	3.46
16	3.22
17	6.15
18	5.70
19	4.00
20	5.52

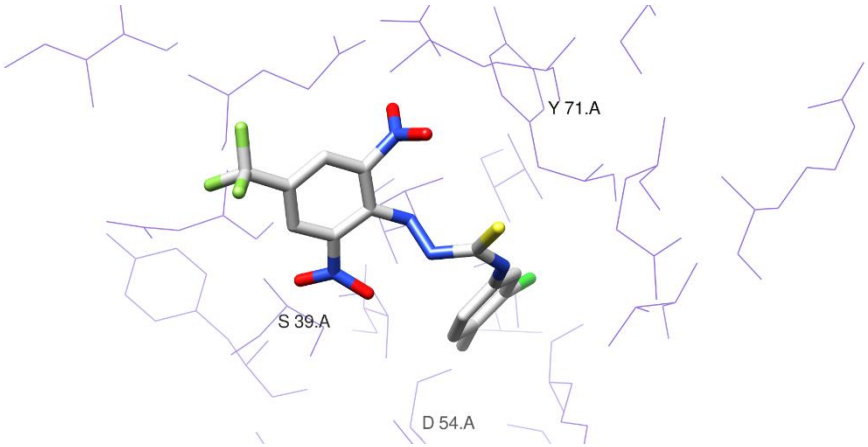
RISULTATI MODELLO PRELIMINARE COMBINE

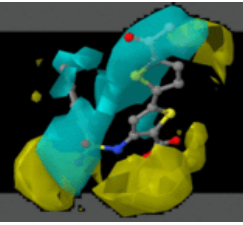
PROBE	PC	r^2	q^2LOO
STE	4	0,807	0,324
DRY	4	0,818	0,276
ELE	2	0,424	0,152
HB	1	0,201	-0,128
STE-DRY	5	0,883	0,299
STE-ELE	5	0,899	-0,044
STE-HB	1	0,230	-0,131
DRY-ELE	2	0,582	0,209
DRY-HB	3	0,735	0,207
ELE-HB	1	0,251	-0,141
STE-DRY-ELE	2	0,630	0,170
STE-DRY-HB	1	0,357	-0,050
STE-ELE-HB	2	0,692	0,036
DRY-ELE-HB	1	0,269	-0,166
STE-DRY-ELE-HB	2	0,709	0,018

COMBINE RISULTATI









CONCLUSIONE

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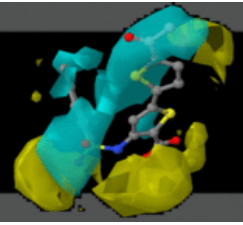
STUDIO *STRUCTURE-BASED* DI *DOCKING* MOLECOLARE

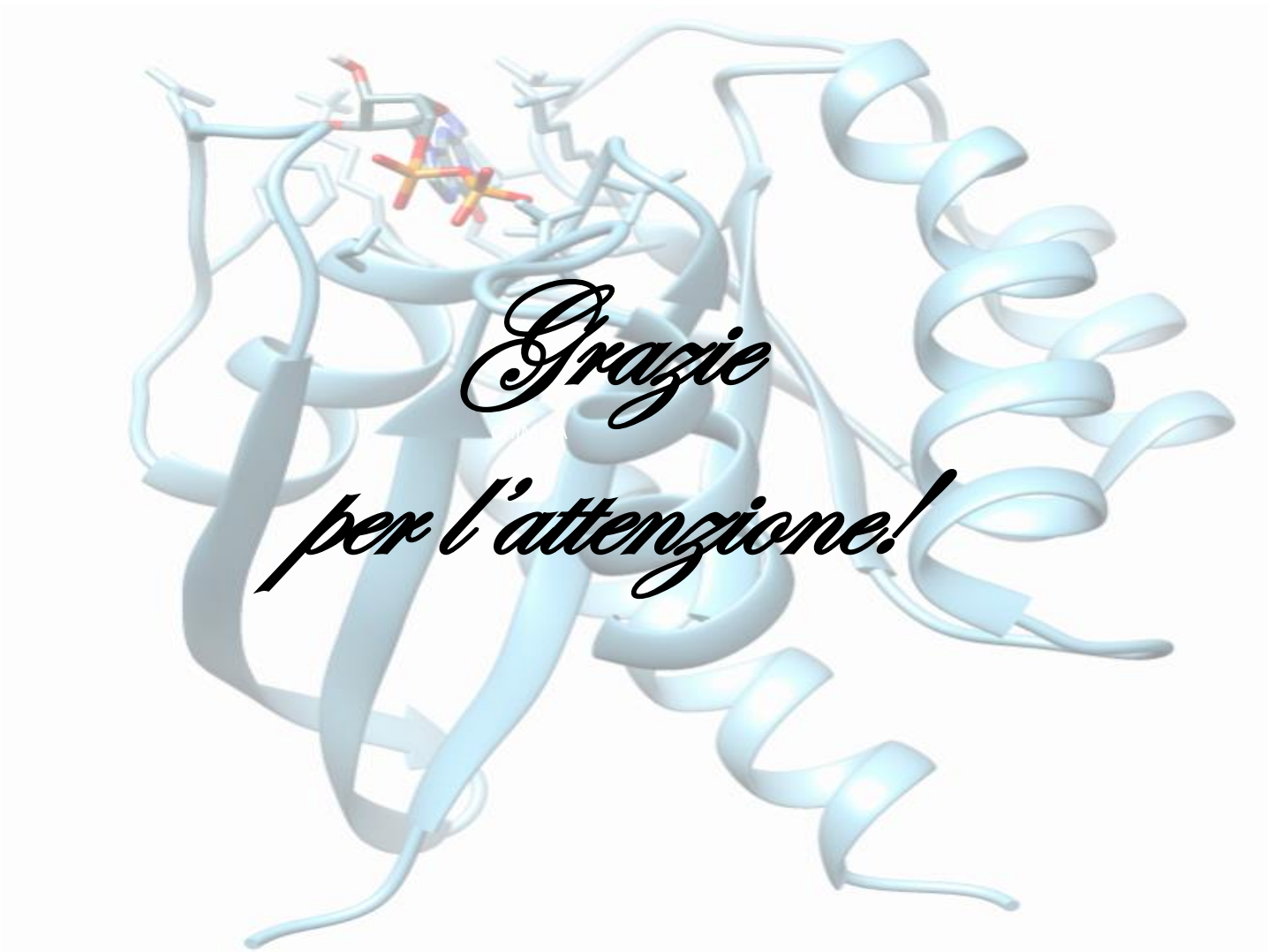
CREAZIONE MODELLO 3-D QSAR

STUDIO COMBINE



OBIETTIVI FUTURI

- VIRTUAL SCREENING
- AMPLIARE IL NUMERO DI MOLECOLE
- CREARE MODELLI PIU' GENERALIZZATI E GLOBALI
- PROGETTAZIONE DI NUOVI INIBITORI



Grazie

per l'attenzione!